



## Long-term impacts of organic and conventional farming on the soil microbiome in boreal arable soil

Krista Peltoniemi<sup>a,\*</sup>, Sannakajsa Velmala<sup>a</sup>, Hannu Fritze<sup>a</sup>, Riitta Lemola<sup>b</sup>, Taina Pennanen<sup>a</sup>

<sup>a</sup> Natural Resources Institute Finland (LUKE), Natural Resources, Latokartanonkaari 9, FI-00790, Helsinki, Finland

<sup>b</sup> Natural Resources Institute Finland (LUKE), Natural Resources, Tietotie 4, FI-31600, Jokioinen, Finland

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### ABSTRACT

Long-term effects of organic and conventional farming systems in parallel on the microbiota of boreal arable soil from forage and cereal crop fields were investigated. Microbial activity was measured as basal respiration and microbial biomass C and N were determined by fumigation extraction. Microbial abundance was determined by gene copy numbers from bacterial and archaeal specific 16S rRNA genes and the fungal ITS2 region with quantitative PCR. Microbial community composition for soil bacteria and fungi, including arbuscular mycorrhiza, were conducted by amplicon sequencing with richness assessed from OTU reads. We detected changes in both bacterial and fungal community composition between the farming systems. Microbial activity and biomass C and N were higher in the organic system for cereal crop rotation compared to the respective conventional system. In the autumn, organic systems had higher microbial richness. As fungi were more abundant in the autumn, they may be responsible for both higher microbial activity and C sequestration in their biomass after harvesting, especially in the organic system for cereal crop rotation. Also, crop type and cow manure explained changes in fungal community composition. The typical bacterial community of the organic system for cereal crop rotation included many soil and plant health promoting bacterial groups. Fungi benefiting from organic farming practices, other than manure, may include endophytic taxa with a variety of functions as well as pathogenic and mycotoxin producing species. Overall, the results suggest that farming practices typical of organic farming, such as use of green manure and continuous plant cover have induced changes in the soil microbiome.

### 1. Introduction

Quite alarmingly, carbon (C) storage of agricultural soil has been reported to decrease by 31% during the first decade after conversion into farmland due to land-use change and to further diminish to less than 50% in half a century [1], accompanied by losses in crop yield [2]. A similar trend was detected from Finnish cultivated fields where the average C loss was 17% over a 35-year cultivation period, and the authors suggested that the change in management practices in recent decades towards increasing cultivation of annual crops, as well as climate change, has contributed to soil C losses in boreal cultivated soils [3].

Intensive soil cultivation causes leaching of essential nutrients and physical damage to soil structures, eventually negatively changing ecosystem services provided by the soil microbiome. Microbiome soil ecosystem services include decomposition, formation of soil aggregates, cycling of nitrogen (N), aid in nutrient and water uptake by plants, pathogen control, mitigation of greenhouse gases and C sequestration

into soil as microbial bio- and necromass [4,5]. For instance, mould-board ploughing, which is used as a common tillage practice in conventional intensive cultivation has been reported to cause many physical, chemical and biological changes in soil including reduced abundance and diversity of soil organisms [6].

Organic cultivation systems may provide beneficial solutions to current problems affecting the soil microbiome. According to a large meta-analysis study, microbial biomass C and N were on average 41% and 51% higher in organic systems, respectively, as well as having 59% increased total phospholipid fatty acids (PLFAs) and 32–84% increased enzyme activities, compared to conventional systems [7]. According to another meta-analysis based on an extensive literature review, organic farming generally also had positive impacts on the biodiversity of soil microbiota [8]. Several studies have reported on the increase of microbial diversity or changes in community composition due to organic farming [9–14]. In addition, organic farming has been reported to lower soil-derived plant diseases [15–17] and increase disease suppressiveness

\* Corresponding author.

E-mail address: [krista.peltoniemi@luke.fi](mailto:krista.peltoniemi@luke.fi) (K. Peltoniemi).

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[18,19]. Organic fertilizers are reported to induce changes in soil microbial communities [20–22] that would promote soil health. According to EC-regulation [23], organic crop rotations are not allowed to have cereals more than three consecutive years, and more than 30% of crop rotation plants must be legumes. Furthermore, synthetic fertilizers and pesticides are prohibited in organic farming. However, management practices used in organic farming e.g., organic fertilizers and more diverse crop rotations are utilized more and more in conventional farming approaching the idea of an integrated farming system [24].

Furthermore, land use intensification differently affects bacteria, archaea and fungi [25]. Microbial communities respond differently to management practices such as tillage intensity [13]. Symbiotic arbuscular mycorrhizal fungi (AMF) colonizing many crop plant roots particularly benefit from management practices used commonly in organic farming, such as diverse crop rotation and reduced tillage [26, 27]. The microbial community composition is important since the soil fungal diversity is suggested to be an intrinsic factor in the health of managed soils [28].

A broad and comprehensive scientific understanding on the long-term impacts of organic farming on soil processes and microbial communities is currently incomplete. Indeed, we are not aware of any previous studies that have been conducted in arable soils of the boreal region and in parallel for forage and cereal crop rotation. In this study, the microbiome was investigated both in spring and autumn sampling campaigns, since temporal factors are known to strongly affect microbial communities, especially in agricultural soils [29]. We used basal respiration as a measure of microbial activity, quantitative PCR (qPCR) for estimating microbial abundance, fumigation extraction to estimate microbial biomass derived C and N, and target gene region amplicon sequencing to reveal microbial community composition. Our aim was to detect whether long-term organic and conventional farming have induced changes in microbial (i) activity, (ii) biomass, (iii) richness, as well as (iv) community composition according to their respective farming practices, and we expected that the direction and magnitude of putative changes are dependent on (v) crop type (cereal vs forage crop), (vi) season, and (vii) the specific microbial group investigated.

## 2. Material and methods

### 2.1. Experimental site

The sampled experimental field site (2.6 ha) is located on sandy soil in Toholampi; Ostrobothnia, western Finland (63.49°N, 24.09°E). The site was constructed for erosion and nutrient leaching studies as described by Turtola and Kemppainen in 1998 [30]. Four different crop rotations in total were established at the field in 2001 to compare conventional and organic farming systems for hypothetical cereal and milk production farms with four-year crop rotations (Supplementary Fig. S1). The main focus of this study was the effect of different nitrogen sources and fertilization intensities on nitrogen leaching and crop yields. The experiment was designed as a randomized block design consisting of 16 plots (size 100 × 16 m) including four replicates for each crop rotation. Organic crop rotations and farming practices were designed to meet the EU requirements for organic farming in terms of crop rotation, fertilization and plant protection [23]. Fertilization was based on the biological nitrogen fixation (BNF) of legumes and use of cattle manure applied as a slurry. Plant protection was executed proactively through crop rotation and tillage.

Organic crop rotation producing cereals (OCer) was planned to cooperate with a dairy farm which provided manure in return for silage. During the entire 18-year period the OCer rotation received on average 50 kg ha<sup>-1</sup> a<sup>-1</sup> total nitrogen (Tot-N) in manure, applied in the first and last year of the four-year crop rotation (last time spring 2017, a year before soil sampling). Organic crop rotation of the milk production farm (OMilk) cultivating forage crops was planned to be self-sufficient in fodder and manure. The crop rotation produced fodder to feed the dairy

cows. The manure produced by the cows was used as organic fertilizer. Average manure application rate during 2001–2018 was 85 kg Tot-N ha<sup>-1</sup> a<sup>-1</sup>. Since 2005 manure has been applied annually.

Conventional cereal crop rotation (CCer) was fertilized with synthetic fertilizers according to the limits of the Agri-Environmental Program in Finland (AEP) and was approximately 86 kg Tot-N ha<sup>-1</sup> a<sup>-1</sup>. Conventional forage crop rotation of a dairy farm (CMilk) was fertilized with a manure application rate of 110 kg Tot-N ha<sup>-1</sup> a<sup>-1</sup>. Annual fertilization with applied manure was complemented with synthetic fertilizers according to the limits of AEP and was about 61 Tot-N kg ha<sup>-1</sup> a<sup>-1</sup>. Fertilization practices during the experimental years 2001–2018 are presented in detail in Supplementary Table S1. In addition, field plots under the conventional systems (CCer, CMilk) received plant protection agents for weed control presented in Supplementary Table S2.

Cultivated plants in the four-year crop rotations were: OCer) barley (*Hordeum vulgare* L.) with undersown ley seed, ley, rye (*Secale cereale* L.) and oats (*Avena sativa* L.); OMilk) barley with undersown ley seed, ley, ley, and mixture of oats and common vetch (*Vicia sativa* L.); CCer) barley, barley, rye and oats; CMilk) barley with undersown ley seed, ley and barley. In organic crop rotations leys were a mixture of timothy (*Phleum pratense* L.) and red clover (*Trifolium pratense* L.), while in conventional crop rotation ley was a mixture of timothy and meadow fescue (*Festuca pratensis* Huds.). Thus, the sampling year 2018 was the second year of the 4-year crop rotation and cultivated plants in rotations were timothy and clover ley for OCer and OMilk, barley for CCer and timothy and meadow fescue ley for CMilk. However, in 2017, the year preceding spring sampling in 2018, barley was cultivated as a main crop in all four rotations.

### 2.2. Soil sampling and nutrient levels

Two soil sampling campaigns were conducted in spring and autumn 2018: first in May before sowing and the second in August after the harvest. One top 20 cm soil layer composite sample per plot was obtained by combining five subsamples taken in lines every 20 m across the whole plot area. The fresh composite sample was divided into three subsamples and kept either under +4 °C for basal respiration analyses or frozen at –20 °C for fumigation extraction and DNA based analyses. Fresh pre-weighted soil was dried at +105 °C for 12 h and cooled in a desiccator before weighing. All results are calculated per dry mass weight.

Nutrient levels for phosphorus (P), calcium (Ca), potassium (K) and magnesium (Mg) from all plots were determined in separate samples from 0 to 10 cm soil layer in October 2018 (Supplementary Table S3). Nutrients were analyzed from soil samples with acid ammonium acetate extraction (HAAC) [31], at the laboratory of Eurofins (Mikkeli, Finland) according to accredited standard methodology (SFS EN ISO/IEC 17025:2005 FINAS T096). The method is routinely used as an advisory soil test in Finland.

### 2.3. Basal respiration, pH measurements and microbial biomass

The soil samples were kept at +14 °C for two days prior to the basal respiration (BR) measurements. The basal respiration rate was determined from freshly measured soil (with a standardized volume-based measuring scoop of 20 ml), sealed with a rubber stopper in a 125 ml infusion bottle, measured for the amount of CO<sub>2</sub> evolved after 24 h incubation and measured as described by Pietikäinen and Fritze in 1995 [32]. Thereafter, soil pH was determined in distilled water (1:3.5, vol/vol) from the same sample. The amount of C and N in the microbial biomass of the soil samples was determined by chloroform fumigation-extraction (FE) as described by Törmänen et al., in 2018 [33]. C and N flushes from the microbial biomass were determined as the difference between the fumigated and non-fumigated samples and converted to microbial biomass C (C<sub>MB</sub>) and microbial biomass N (N<sub>MB</sub>) as mg of C or N kg<sup>-1</sup> (dry soil). Non-fumigated K<sub>2</sub>SO<sub>4</sub> extractable C and N

correspond to labile forms of soil C ( $C_{EXT}$ ) and N ( $N_{EXT}$ ).

#### 2.4. DNA extraction, qPCR and amplicon sequencing

Soil DNA was extracted with a NucleoSpin Soil kit (Macherey Nagel, Germany) according to the protocol of the manufacturer from homogenised soil samples from which all roots were removed. DNA concentration and purity were determined with a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific). DNA samples were sequenced at the Institute of Genomics of Tartu University, Estonia. AMF fungi were sequenced from four composite DNA samples (CMilk, CCer, OCer, and OMilk) obtained from autumn samples with AMF targeting the 18S rRNA gene using primers AML2 [34] and universal eukaryotic primer WANDA [35]. For bacteria the targeted V4 region of the 16S SSU rRNA and for fungi the ITS2 region were amplified in a two-step polymerase chain reaction (PCR). Bacterial and fungal PCR were performed using the 16S rRNA primers 515F and 806R [36,37] and the ITS primers ITS4 [38] and gITS7 [39], respectively, with 8bp dual index for 24 cycles. The final PCR fragments were run as paired-end  $2 \times 300$  bp with the MiSeq platform (Illumina) using MiSeq v3 kit producing ca. 20–25 M reads per flow cell. Quantitative PCR for the partial fungal ITS region, and bacterial and archaeal 16S rRNA genes, was conducted as described by Peltoniemi et al., in 2015 [40], for all samples separately.

#### 2.5. Filtering of raw sequence reads and bioinformatics

Sequence assembly, quality filtering, removal of artefacts, primer-dimers and primers from raw 16S and 18S rRNA and ITS2 sequence reads, along with clustering and taxonomical annotations were conducted with PipeCraft 1.0 pipeline [41] as described by Soenne et al., in 2020 [42]. In addition, during assembling and quality filtering for 18S rRNA targeting AMF, a minimum length of 200 bp and trunc qual 0 was used. AMF OTUs were taxonomically annotated by searching for representative sequences against MaarjAM database [43] for 18S rRNA, with parameters lastn, max\_target\_seqs 10, evaluate = 0.001, word size = 7, reward = 1, penalty = -1, gapopen = 1, gapextend = 2, outfmt = 5.

After the first quality filtering steps, raw bacterial 16S rRNA sequence data consisted of 915 740 reads clustering into 13 824 OTUs, fungal ITS2 data consisted of 663 744 reads clustering into 2800 OTUs, and raw AMF 18S rRNA sequence data consisted of 157 011 reads clustering into 1328 OTUs. Second quality filtering was done as described by Soenne et al., in 2020 [42]. OTUs that had affiliations other than bacteria or fungi, as well as singleton OTUs and reads with relative proportion below 0.01% were removed from the data. Furthermore, bacterial OTUs were consolidated according to accession numbers in the Silva database [44], fungal OTUs to the exact same species hypothesis in UNITE [45], and AMF OTUs according to genbank accession numbers in MaarjAM database. Only 25% of the 18S rRNA sequences had >90% identity to their reference.

The final bacterial data consisted of 472 119 and 347 709 reads for spring and autumn samples, respectively, clustering into 2110 OTUs. For fungi, the spring and autumn data consisted of 268 238 and 272 290 reads, respectively, and clustered into 1190 OTUs. The final AMF data consisted of 38 940 reads for four bulked autumn samples, clustering into 39 OTUs. Raw sequence data is deposited to the sequence read archive (SRA) of NCBI/EMBL database under the BioProject id PRJNA637213 with the accession numbers SAMN15098534-15098565 for bacterial 16S rRNA and fungal ITS2 data, and SAMN15098881-SAMN15098884 for AMF 18S rRNA data.

#### 2.6. Statistical analyses

All statistical analyses were conducted in R studio version 1.2.5001 and R version 3.6.0 or 3.6.1 [46]. Differences in means of basal respiration rates, soil pH, total soil C and N, microbial biomass C and N, extractable C and N, 16S rRNA gene and ITS-region copy numbers, and

bacterial and fungal OTU numbers (richness), between the farming systems (organic vs conventional), and for the spring and autumn data separately, were investigated with function lmer (package lmerTest) producing a linear mixed model that takes into account the impact of the block design [47]. The significance of the linear model was tested with type III analysis of variance with Kenward-Roger's method [48] with function anova. Pairwise analyses and significant differences of farming systems were tested with function lsmeans (package lsmeans) (alpha  $p < 0.05$ ) [49]. OTU data from the amplicon sequencing was normalized using the geometric mean of pairwise ratios (GMPR) method [50]. We performed permutational multivariate analysis of variance (PERMANOVA) using distance matrices with function adonis from vegan 2.5–5 [51] to test the effect of farming system and crop type (cereal vs forage), and the effect of manure addition, including plants grown in 2018, on microbial community composition. We also conducted 2-D nonmetric multidimensional scaling (NMDS) with stable solution from random starts, axis scaling and species scores with function metaMDS from vegan using the Bray-Curtis dissimilarity index and plotted the NMDS with fitted environmental variables (soil pH; basal respiration; total, extractable and microbial biomass C and N; bacterial 16S rRNA and fungal ITS-region copy numbers; concentrations of P, Ca, K and Mg) from function envfit in vegan. Fungal and bacterial OTUs indicative for specific rotations were obtained by differential abundance analysis (DESeq2) which identified significant groups ( $>|1.7|$  log<sub>2</sub> fold change with adjusted  $p < 0.05$  or  $<0.001$ , for bacteria and fungi) [52]. Due to the PERMANOVA results we also did additional differential abundance analyses to obtain OTUs indicative for rotation plots that have either received manure or not. The results are presented as paired comparisons between the farming systems for forage and cereal crop rotations, and plots with manure or not, and for the spring and autumn data separately.

### 3. Results

#### 3.1. Basal respiration, soil pH and estimated microbial biomass

Basal respiration (BR) and microbial biomass C ( $C_{MB}$ ) and N ( $N_{MB}$ ) amounts were significantly higher in the OCer rotation compared to the CCer rotation both in the spring and autumn data (Table 1). Soil pH was also higher in the OCer rotation, but only in the autumn data. In contrast, BR rate and  $C_{MB}$  were clearly higher in the CMilk rotation compared to the OMilk rotation in the spring data. Total or extractable C ( $C_{EXT}$ ) and N ( $N_{EXT}$ ) did not differ between the farming systems for either cereal or forage crop rotations.

#### 3.2. Microbial richness, copy amounts and community composition

Bacterial richness, assessed by OTU reads, was significantly higher in the organic rotations (OCer and OMilk) compared to the conventional rotations (CCer and CMilk) in the autumn data (Table 2). Furthermore, fungal richness was higher in the organic systems in the autumn data but only significantly for the OMilk rotation. In the spring data bacterial and fungal richness did not differ between farming systems. Neither did bacterial and archaeal 16S rRNA gene copy numbers differ between farming systems in either the spring or autumn data, whereas fungal ITS copy numbers were higher in the OCer rotation compared to the CCer rotation in the autumn data (Table 2).

The NMDS ordination showed minor differences in bacterial community composition between the farming systems for both crop rotations (Fig. 1a and b). Differences in bacterial community composition, however, were more distinct in the autumn data and higher basal respiration, microbial biomass C and N, as well as extractable C and N variables fitted best with the CMilk rotation. Whereas changes in the fungal community composition between farming systems were more pronounced for both crop rotations and between the seasons (Fig. 1c and d). The fungal community composition in the organic rotations were more similar than between the conventional rotations. In the spring

**Table 1**

Means of soil pH, basal respiration (BR) as mg CO<sub>2</sub> produced kg<sup>-1</sup> (dry mass of soil), total soil C (C<sub>TOT</sub>) and N (N<sub>TOT</sub>) as g kg<sup>-1</sup> (dry mass of soil), microbial biomass derived carbon (C<sub>MB</sub>) and nitrogen (N<sub>MB</sub>), extractable soluble carbon (C<sub>EXT</sub>) and nitrogen (N<sub>EXT</sub>) as g kg<sup>-1</sup> (dry mass of soil), obtained from samples originating from plots (n = 4) with different productions systems for cereal and forage crop rotation plots sampled in the spring and autumn. Standard error of means in parentheses. Letters show the significant differences between means tested in anova (*p* < 0.05).

Season	Plot	soil pH	BR	C <sub>TOT</sub>	C <sub>EXT</sub>	C <sub>MB</sub>	N <sub>TOT</sub>	N <sub>EXT</sub>	N <sub>MB</sub>
spring	OCer	6.35 (0.05)a	39.5 (1.6)bc	4.42 (0.29)a	0.037 (0.001)a	0.14 (0.01)b	0.17 (0.01)ab	0.021 (0.006)a	0.019 (0.009)b*
	OMilk	6.23 (0.06)a	38.8 (4.7)ca	4.77 (0.37)a	0.045 (0.001)a	0.14 (0.01)b	0.19 (0.01)c	0.023 (0.005)a	0.016 (0.002)b*
	CCer	6.28 (0.05)a	30.9 (3.8)a	4.22 (0.24)a	0.041 (0.003)a	0.09 (0.004)a	0.16 (0.01)a	0.012 (0.002)a	0.012 (0.001)a*
autumn	CMilk	6.23 (0.05)a	47.7 (1.1)b	4.96 (0.58)a	0.042 (0.002)a	0.17 (0.01)c	0.19 (0.01)bc	0.018 (0.003)a	0.024 (0.002)ab*
	OCer	6.43 (0.02)b	44.0 (3.7)b	4.34 (0.39)ac	0.057 (0.004)ac	0.15 (0.01)b	0.17 (0.01)ac	0.011 (0.001)ac	0.021 (0.001)bd
	OMilk	6.25 (0.03)c	50.8 (4.5)cb	4.62 (0.44)bc	0.062 (0.002)bc	0.15 (0.01)bc	0.19 (0.01)bc	0.014 (0.001)bc	0.023 (0.002)cd
	CCer	6.10 (0.04)a	21.6 (1.2)a	3.95 (0.18)a	0.051 (0.001)a	0.09 (0.01)a	0.15 (0.003)a	0.011 (0.0005)a	0.013 (0.001)a
	CMilk	6.25 (0.06)c	45.3 (1.9)cb	5.11 (0.64)b	0.063 (0.002)bc	0.17 (0.01)c	0.20 (0.02)b	0.017 (0.002)b	0.027 (0.001)c

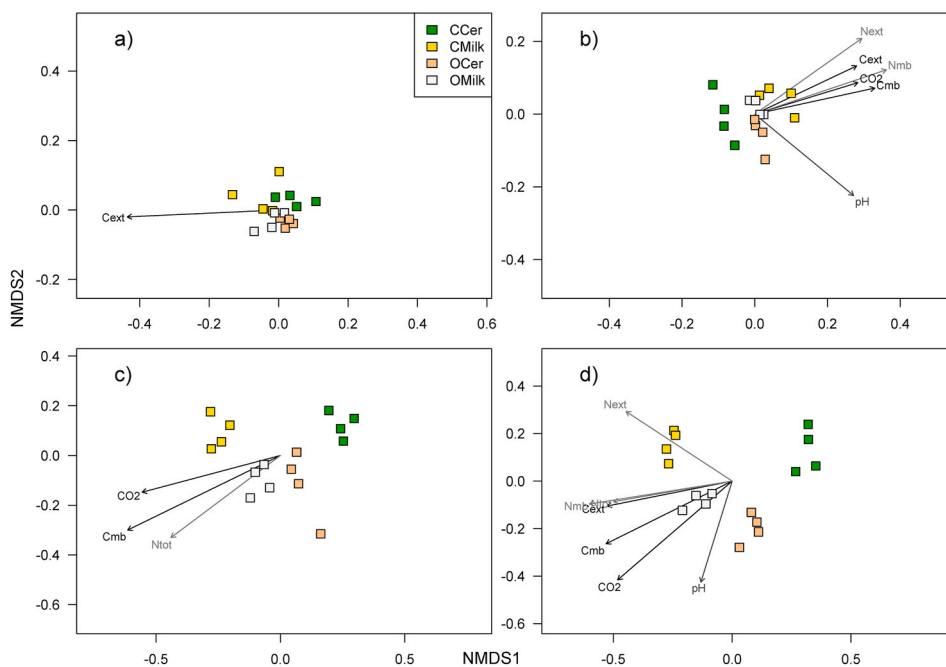
Abbreviations: C; conventional system; O, organic system; Cer, cereal crop rotation; Milk, forage crop rotation. \* Box-Cox transformed data.

**Table 2**

Mean OTU numbers (richness) obtained from GMPR transformed data for bacteria and fungi, and means of partial bacterial and archaeal 16S rRNA gene and fungal ITS2 region copy amount as copies g<sup>-1</sup> (dry mass of soils) obtained from the crop rotation plots (n = 4) of the different farming systems sampled in the spring and autumn. Standard errors of means are shown in parentheses. Letters show the significant differences between means tested in anova (*p* < 0.05).

Season	Plot	bact OTU numbers	fung OTU numbers	bact 16S rRNA copies	arch 16S rRNA copies	fung ITS2 copies
spring	OCer	1953 (13)a	397 (13)a	1.3E+10 (9.1E+08)a	2.4E+07 (3.1E+06)a	3.7E+08 (4.8E+07)a
	OMilk	1935 (38)a	371 (17)a	1.4E+10 (8.7E+08)a	3.3E+07 (4.1E+06)a	3.9E+08 (3.2E+07)a
	CCer	1901 (26)a	374 (9)a	1.4E+10 (1.2E+09)a	3.7E+07 (8.2E+05)a	2.7E+08 (4.9E+07)a
	CMilk	1867 (60)a	360 (20)a	1.2E+10 (2.3E+09)a	2.8E+07 (6.0E+06)a	3.9E+08 (1.0E+08)a
autumn	OCer	1820 (47)bc	391 (9)a	9.3E+09 (3.1E+08)a	2.3E+07 (3.9E+06)ab	6.5E+08 (7.9E+07)b
	OMilk	1854 (10)b	404 (9)ac	9.4E+09 (2.5E+08)a	2.2E+07 (2.7E+06)ab	7.9E+08 (3.4E+07)c
	CCer	1721 (30)a	372 (23)ab	8.6E+09 (5.9E+08)a	2.6E+07 (2.4E+06)a	3.6E+08 (3.8E+07)a
	CMilk	1734 (41)ac	344 (15)b	9.9E+09 (6.5E+07)a	1.9E+07 (1.7E+06)b	7.4E+08 (7.4E+07)cb

Abbreviations: see Table 1.



**Fig. 1.** Non-metric multidimensional scaling (NMDS) analysis for GMPR transformed bacterial 16S rRNA a) spring and b) autumn data and fungal ITS2 c) spring and d) autumn data separately. Vectors show significant environmental factors for measured variables in ordination (*p* < 0.05). Abbreviations: CCer, conventional systems for cereal crop rotation; OCer, organic system for cereal crop rotation; CMilk, conventional system for forage crop rotation; OMilk, organic system for forage crop rotation.

data, higher basal respiration rates and microbial biomass C and N variables fitted best with the OMilk rotation. In autumn data, microbial C and N, and extractable C fitted best with the OMilk rotation, and soil pH and basal respiration rates with the OCer rotation, and extractable N with the CMilk rotation.

The first PERMANOVA indicated that both farming system and crop type significantly affected the variation in microbial community composition both in the spring and autumn data. However, crop type did

not explain the variation in the bacterial community composition in the spring data (Table 3a). Farming system explained 10 and 14% of the variation in bacterial community composition in the spring and autumn data, respectively (Table 3a). Crop type explained 10% of the variation in the autumn bacterial data. In turn, the farming system explained 11 and 14% of the variation in fungal community composition in the spring and autumn data, respectively. Crop type explained even more, 21 and 36% of the variation, in the spring and autumn data, respectively.

**Table 3**

Results of two PERMANOVA analysis to test a) the effect of organic or conventional farming systems (System) and cereal and forage crops (Crop type) and also b) the effect of manure including crop plant in 2018 (Manure/Plant2018) on microbial OTU composition in the spring and autumn data. Differences are considered significant if  $p \leq 0.05$ .

Source	Spring			Autumn		
	F	R2	P	F	R2	P
<b>Bacteria</b>						
a) System	1.625	0.102	0.02	2.310	0.135	0.003
Crop type	1.187	0.075	0.14	1.784	0.104	0.03
Residuals	0.822			0.760		
b) System	1.639	0.103	0.01	2.376	0.135	0.003
Crop type	1.197	0.075	0.12	1.834	0.104	0.03
Manure/Plant2018	1.113	0.069	0.18	1.37	0.077	0.11
Residuals	0.752			0.682		
<b>Fungi</b>						
a) System	2.159	0.112	0.05	3.536	0.136	0.02
Crop type	4.100	0.212	0.001	9.302	0.360	0.001
Residuals	0.675			0.503		
b) System	2.491	0.112	0.03	4.209	0.136	0.01
Crop type	4.731	0.212	0.001	11.07	0.360	0.001
Manure/Plant2018	3.001	0.135	0.01	3.471	0.112	0.02
Residuals	0.539			0.390		

We also tested the effect of manure in an additional PERMANOVA test, which showed that in addition to farming system manure did not affect the variation in bacterial community composition (Table 3b). However, manure explained additional 14% and 11% of the variation in fungal community composition in the spring and autumn data, respectively. In addition, the impact of manure could not be separated from the impact of crop plant (timothy) which was grown in all manure fertilized rotation plots in 2018.

### 3.3. Arbuscular mycorrhizal fungal community composition

In the ITS2 based fungal data we could identify on average from 9 to 15 Glomeromycotan AMF OTUs in the spring, and from 12 to 25 in the autumn. In the spring data, the OMilk rotation had the lowest (9) OTU number and the OCer rotation the highest (14). In the autumn data, the CMilk rotation had the lowest (12) and the OCer rotation the highest (25) AMF OTU number. The majority of AMF OTUs were shared between the farming systems. PERMANOVA showed that season explained the most variation (14%) in the AMF community composition, while crop type (8%) and farming system (6%) also had an effect. There were large differences in the relative abundances of AMF families between the spring and autumn data of the organic systems (Supplementary Fig. S2). *Claroideoglomeraceae* was the most abundant family, especially in the autumn data, whereas *Acaulosporaceae* and *Pacisporaceae* were not present at all in the CMilk rotation. *Archaeosporaceae* were abundant in the organic systems and almost absent from the conventional systems. It also seems that *Pacisporaceae*, while not abundant, is characteristic to the cereal rotations, regardless of the season.

The 18S rDNA-based AMF data from composite samples obtained in the autumn identified 38 OTUs with unique genbank accession numbers (Supplementary Table S4). Moreover, two *Scutellospora* species were obtained from the *Gigasporaceae* family that were not seen in the ITS2 data (Supplementary Fig. S2). *Paraglomeraceae*, *Claroideoglomeraceae* and *Diversisporaceae* were the most abundant AMF families based on the 18S data. Organic systems had higher AMF richness; AMF OTU numbers varied from 22 in soil with conventional systems to 35 in soil from OMilk. One out of two *Acaulospora* sp. and four out of seven *Glomus* sp. OTUs were only present in the organic systems, with two of them only in the OMilk rotation. Altogether, 19 OTUs were shared between all four rotations. *Glomus* species were abundant in the OMilk rotation, and similarly to the ITS2 based data, *Pacisporaceae* was not present in the CMilk rotation, but we could find one *Acaulosporaceae* OTU present in all

rotations, contrary to the ITS2 data. Cereal systems were characteristically associated with *Archaeospora trappei*, *Archaeospora* sp, *Glomus mosseae*, and *Pacispora* sp. (Supplementary Table S4).

### 3.4. Bacterial OTUs for the farming systems and crop rotations

In general, there were only two bacterial OTUs typical of the forage crop rotation. The first OTU was affiliated to genus *Altererythobacter* (Sphingomonadaceae) and it was typical of the conventional system. The second OTU was affiliated with the uncultured S0134 terrestrial group of Gemmatimonadetes that was more typical of the organic system. Both appeared in the spring data. Moreover, there were 28 and 12 indicative OTUs for the cereal rotations (OCer and CCer), respectively (Supplementary Table S5). In addition, there were more indicative bacterial OTUs detected in the autumn data compared to the spring data.

Indicative OTUs for the spring and autumn data in the CCer rotation were those affiliating to Proteobacteria and Gemmatimonadetes (genera *Rhodanobacter* and *Gemmatimonas*). In the spring data, indicative OTUs for CCer affiliated to the genus *Anaeromyxobacter* and family *Moraxellaceae*, and in autumn data OTUs to genera *Burkholderia*, *Elusimicrobia*, *Gemmatimonas*, *Granulicella*, *Mucilaginibacter*, *Planifilum* and the family *Chitinophagaceae*.

Irrespective of the season, the OCer rotation was characterized by OTUs that affiliated to three phyla (Actinobacteria, Firmicutes, Proteobacteria) including eight genera (*Actinocorallia*, *Bacillus*, *Cupriavidus*, *Fodinicola*, *Lysobacter*, *Mycobacterium*, *Nannocystis*, *Rummeliibacillus*). Whereas in the spring data indicative OTUs for the OCer rotation affiliated to Firmicutes, Proteobacteria and Bacteroidetes including five genera (*Bosea*, *Clostridium*, *Dyadobacter*, *Pedobacter* and *Sphingomonas*), and in the autumn data to Actinobacteria, Firmicutes, Proteobacteria and Planctomycetes including five genera (*Iamia*, *Nocardioides*, *Romboutsia*, *Streptosporangium* and *Turcibacter*).

### 3.5. Fungal OTUs for the farming system and crop rotations

Generally, more indicative fungal OTUs were detected from the organic than from the conventional systems (Supplementary Table S6). As for bacteria, there were far fewer indicative fungal OTUs in forage compared to cereal crop rotation. There were a few indicative OTUs that were common only in the spring or autumn data and in conventional or organic systems. For instance, an OTU affiliating to the ascomycetous *Oidiodendron echinulatum* was typical of the OMilk, as well as the CCer rotations. The OTUs affiliating to *Arthrinium serenense* and *Cyathicula culmicola* were more typical of the OMilk rotation in the spring data and of the OCer rotation in the autumn data. In the spring data both conventional rotations were characterized by only two OTUs that were affiliated to the basidiomycetous fungal family *Ceratobasidiaceae* and species *Lachnella villosa*.

OTUs typical of the OMilk rotation in the spring data were those affiliated to *Gibellulopsis piscis* and *Pleosporeles* sp., and both in the spring and autumn data the OTU affiliated to *Ilyonectria mors-panac*. In the autumn data an indicative OTU affiliated to *Fusarium culmorum* was typical of the CMilk rotation.

There were several indicative fungal OTUs in the OCer rotation both in the spring and autumn data. Most of them were affiliated to ascomycetes such as *Pseudaleuria* sp. belonging to the Pezizales, Sordariomycetes, Sordariales, *Lasiosphaeriaceae* and genus *Cladorrhinum*. There were also a few other than ascomycetes in this group which were affiliated to Mucoromycota, Mortierellomycota and basidiomycetous *Solicozozyma phenolica* and *Holtermanniella takashimae*. Fungal OTUs that were affiliated to ascomycetous taxa *Apiosporaceae*, *Orbiliaceae* and basidiomycetous genus *Naganishia* were more typical of the spring data, whereas in the autumn data indicative OTUs for the organic system were affiliated to *Articulospora* sp., *Olpidium brassicae*, *Fusarium oxysporum* and *Glarea lozoyensis*.

Both in the spring and autumn data the CCer rotation had the same

indicative OTU that was affiliated to *Myrmecridium schulzeri*. In autumn indicative OTUs were affiliated to *Oedogoniomyces* sp., *Lophotremataceae* sp., *Vishniacozyma carnescens* and *Mortierella elongata*.

### 3.6. Fungal and bacterial OTUs for rotations with manure fertilization

Almost all (90%) bacterial OTUs typical of the plots that have received manure both in the spring and autumn data represented the same taxa that were typical of the OCer rotation listed above (Supplementary Table S7). However, there were four OTUs that were observed only from the OCer rotation and not in the other manure plots; OTUs representing Proteobacteria and its families *Sandariaceae* and *Beijerinckiacae* (genus *Bosea*) in the spring data, and OTUs representing uncultured Planctomyces and actinobacterial genus *Nocardioidea* in the autumn data.

As for bacteria, the majority of fungal OTUs (80%) typical of rotation plots that have received manure fertilization were the same obtained for the OCer rotation both in the spring and autumn data (Supplementary Table S8). However, there were a few fungal OTUs that were observed only from the OCer rotation and not in the other manure plots; OTUs representing uncultured *Apiosporaceae*, *Helotiales* and *Cyathicula culmicola* in the spring data, and OTUs representing *Arthrinium serenense*, *Fusarium oxysporum*, *Glarea lonoyensis* and *C. culmicola* in the autumn data.

## 4. Discussion

### 4.1. Changes in microbial activity, biomass and community composition between the farming systems

The more frequent tillage in the conventional system for cereal crops explains partly the lower microbial activity rates and biomasses observed in spring and autumn, a phenomenon which has been observed also by others [53–55]. Furthermore, the conventional cereal system had bare soil over the winter and is the only treatment without manure addition in the rotation. Thus, the absence of continuous plant cover may have further reduced the microbial biomass C in the spring compared to the respective organic system as earlier reported [56]. Moreover, the second cut of grass and clover ley was left on the soil surface as a green manure in the organic system for cereal rotation and this may have led to the higher microbial activity and biomass in the following autumn. The lack of chemical agents may also have been reflected in the results since earlier studies have shown negative effects of agrochemicals on soil microbial communities [57]. Higher springtime microbial activity and biomass in the conventional forage crop rotation compared to that of organic cannot be easily explained by the differences in the rotation types as the rotations include various management practices. However, there was 30–40% higher soil P concentration in the conventional system for both cereal and forage crop rotations compared to the organic ones. Indeed, lower water soluble and inorganic P amounts have been reported from organic systems compared to systems receiving synthetic fertilization in a long-term field experiment [58]. Possibly the springtime bacterial community in the conventional forage crop rotation gained competitive advantage from the higher availability of P, since P has been reported to limit bacterial growth in agricultural soils [59]. Later in autumn, the summertime amendments of cow manure would equalize the P availability, and differences between organic and conventional systems for forage crop rotation are no longer detected.

The slightly higher autumn pH in the organic system for cereal crop rotation may result from microbial decomposition of fresh plant residues with high N content and mineralization of ammonium which temporarily is known to increase pH [60]. Furthermore, it has been reported that long-term application of manure maintains the soil pH, but inorganic fertilizer decreased it [61]. Consequently, since bacterial growth is known to increase multifold with increasing pH [62], this may explain

the increased microbial biomass in the organic system for cereal crop rotation. Alternatively, the increased fungal abundance in the organic system for cereal crop rotation in the autumn indicated that fungi could also be responsible for the higher respiration activity and sequestration of C into their biomass. Moreover, the organic system for cereal crop rotation had timothy and clover ley as the main crop plant in the sampling year instead of barley as in the respective conventional system. Indeed, higher microbial biomass in production systems including ley grasses have been detected compared to single crop systems only [63].

Our results showed that the farming system induced a clear shift in microbial community composition and that the overall impact of the farming system was about the same magnitude for both bacterial and fungal community composition. Our results are comparable to previous findings that about 10% of variation in microbial communities was explained by the farming practices of conventional and organic systems [13]. Yet, crop type affected fungal community composition in particular, especially in the autumn. A simple explanation would be that changing cultivated plants from barley in the year 2017 to ley in the sampling year 2018 induced a shift in fungal community composition. Furthermore, it is likely that the summertime amendments of synthetic fertilization in the conventional systems have also contributed to the lowered bacterial and fungal richness in the autumn, since the quality of fertilizer is known to impact largely on microbial communities [20]. Thus, the differences in crop rotation, tillage and fertilization practices (synthetic or organic fertilizers) may all have contributed to the differences in the microbial community but with the current experimental layout we are not able to determine which practices have the strongest impact. Nevertheless, comparisons within the cereal rotations were valuable for indicating the long-term impacts of manure addition and overwintering as bare soil, while comparing the forage crop rotations it was possible to assess the other effects of organic practices beyond manure addition and undersown ley.

Moreover, the differences in AMF richness between the farming systems were only moderate and non-existing under the cereal crop rotation. A higher diversity of *Acaulospora* species, typical to organic systems [64] was also supported by our study. We did not observe *Clareideoglossum* species to be characteristic to organic systems, instead finding the opposite, which contradicts previous results [65]. Thus, our study supports previous observations [66,67] which concluded that mycorrhizal diversity is not influenced by the farming system but rather cultivation practices and conditions, and that finding a universal AMF indicator for farming systems is not feasible.

### 4.2. Differences in specific microbial representatives due to farming system and crop rotation type

Results suggest that the cow manure applied in the forage crop rotation under both the conventional and the organic systems over the years has shaped the bacterial and fungal communities more than any other farming system specific practice, since only a few representatives were typical of either farming system. This also highlights the commonness of pathogenic fungi in the fields cultivated for fodder and fertilized with manure irrespective of the farming system. On the contrary, AMF richness in the forage crop rotation varied clearly due to the farming system; for instance, *Glomus* species were indicative in the organic system, while *Pacispora* sp. was totally missing from the conventional system. Since plants acquire P directly and through their symbiotic AMF [68], the 40% higher levels of P in the conventional forage crop rotation compared to the respective organic rotation may partly explain the lower AMF richness [69].

However, bacterial representatives were less diverse in the conventional farming system of the cereal rotation compared to the respective organic system. Representative taxa typical of the conventional cereal crop rotation in autumn were affiliated to decomposer and plant-growth promoting [70,71] and cellulose decomposing bacterial taxa [72]. In contrast, both the spring and autumn data obtained from the organic

system for cereal crop rotation revealed a variety of specific taxa with diverse functional roles benefiting soil health. Most of these taxa were also linked to manure fertilization, and included for instance, plant growth-promoting rhizobacteria that include species capable of N-fixation, P solubilization, phytohormone production, and repression of soil-borne plant pathogens [73], and genera with antifungal and antibiotic capability [74,75].

Furthermore, the spring and autumn data of the organic system for cereal crop rotation contained also season specific taxa, which shared bacterial representatives with similar functional roles. In addition, these included many beneficial bacteria with antimicrobial features [76], degraders of contaminants and producers of extracellular polymeric substances which are known to improve soil structure and to promote plant growth and drought tolerance [77–80]. In addition, results are comparable with earlier findings that Firmicutes, including well-known pathogenic *Clostridium* species [81,82], are typical of organically managed plots and are most likely linked to manure fertilization [13,14,22,83–85].

In general, actinobacterial representatives were more prominent in the organic system for cereal crop rotation and in rotations with manure fertilization. Indeed, high abundance of actinobacteria have been reported in root samples from organic managed soils [13]. Interestingly, our results showed that actinobacterial genus *Nocardioideis* may have benefited from some other organic system specific practice than manure in the cereal rotation. Indeed, actinobacteria have been found to be indicators for no-tilled organic farming systems, and suggested as producers of exopolysaccharides and lipopolysaccharides, and to have relevance in soil aggregate stability in reduced tillage systems [86]. Furthermore, genus *Bosea* which contains root-nodule endophytic bacteria capable of dinitrogen fixing [87] was specific for the organic cereal rotation system with legumes.

There were fewer changes in fungal representatives in the conventional system for the cereal crop rotation between farming systems compared to changes in bacteria. These fungi included soil saprotrophs [88] and mycoparasites [89] which are general opportunists that either benefit from or tolerate synthetic fertilizers or tilling or both. In general, conditions in autumn may favour fast-growing saprotrophic fungi that effectively make use of harvest residues. Conversely, mycelia of AMF are dependent on living plants but as spores AMF may persist in soil even after harvesting [90]. Here, *Archaeospora trappei* and *Archaeospora* sp., *Glomus mosseae*, and *Pacispora* sp. were indicative mycorrhizal fungi for the cereal crop rotation.

Most of the specific fungi for the organic system for cereal crop rotation were typical of both seasons, indicating certain seasonal stability in the fungal communities in studied arable soils. Furthermore, the majority of these specific fungal representatives were the same as the species specific for the manure fertilized plots. Most of them affiliated to ascomycetes and especially to the order Sordariales. Thus, the indicative fungal representatives in both the organic system for cereal crop rotation and manure fertilized plots consisted of functionally a wide mixture of soil and litter organisms [91,92], including molds and yeasts acting as saprotrophs [93,94], pathogens and predators of other organisms [95].

However, a species of *Arthrinium serenense* was indicative for both organic rotations but not to manure plots, indicating that it could benefit from some other organic farming practice than manure fertilization. Endophytic genus *Arthrinium* has been suggested to have various roles in extreme temperature tolerance, production of substances against other fungi and herbivores, as well as acting saprotrophic and pathogenic [96–99]. Other taxa linked to organic cereal rotation included representatives of *Apiosporaceae* and Helotiales detected in spring, and the pathogenic *Fusarium oxysporum* and its antagonist mycotoxin producing *Glarea lozoyensis* [100] in autumn. These fungi may have the ability to grow quickly and benefit from the second cut of the grass and clover ley which was left on the field as a green manure in the organic system for cereal crop rotation.

## 5. Conclusion

While we could not separate the impact of single management practises on the soil microbiome in the present study, the results provide a view of the long-term effects of systematic practise of these farming systems. Nevertheless, organic systems in both forage and cereal rotations possessed higher microbial richness in autumn, most likely due to a higher variety of organic plant residue substrates after the growing season. Higher availability of P in the conventional system for forage crop rotation may explain higher springtime microbial activity and biomass compared to the organic system. Similar load of cow manure fertilization and continuous plant cover over the years applied in organic and conventional systems of the forage crop rotations may explain minor changes in microbial community compared to the cereal crop rotation. Thus, the results indicate that crop rotation type and whether the rotation includes manure fertilization have an impact on the microbiome in the long-term experimental field site.

Nevertheless, differences between systems, such as tillage conducted in the year preceding the sampling combined with summertime synthetic fertilization in the conventional system for cereal crop rotation, compared to use of green manure, continuous plant cover and organic fertilization in the respective organic system, likely explain most of the results. Organic farming in the cereal rotation increased the abundance of fungi which may be responsible for the higher microbial activity and biomass derived C and N in the autumn. Results indicated that long-term organic farming may facilitate the occurrence of endophytic fungi with broad functional roles as well as common pathogenic fungi and their antagonists. Furthermore, the organic cereal crop rotations seem to promote functional potential within the bacterial community by increasing occurrence of many plant-growth and soil-health promoting bacteria. Interestingly, Lehman et al. [101] concluded that functional complexity of microbes is driving persistence of soil C, and constant soil management practises instead of single attempts are needed to prevent the loss of soil C.

Detecting long-term changes from the microbial perspective may be a challenge for several reasons, e.g., bacteria adapt rapidly to altered conditions. We suggest that by investigating the interactions of soil fauna and microbes we could achieve a more holistic view about the most relevant soil taxa in organic cultivation systems that promote ecosystem services to sustain healthy soil structure, C sequestration and nutrient cycling.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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